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DOCUMENT-IDENTIFIER: US 5853746 A
TITLE: Methods and compositions for the treatment and repair of defects or lesions in cartilage or bone using functional barrier

Brief Summary Text (20):
In general outline, the methods and compositions of this invention for treating superficial cartilage defects or the cartilage portion of full-thickness defects comprise filling the cartilage portion of the defect with a cartilage repair matrix containing an anti-angiogenic agent for inhibiting vascular ingrowth such as anti-invasive factor, metalloprotease inhibitor or antibodies against angiogenesis-inducing factors. The cartilage repair matrix will be incorporated into the animal tissue and is generally biodegradable; it may also contain a proliferation agent and a transforming factor. The cartilage repair matrices of this invention are particularly useful for treating full-thickness defects and apparent superficial cartilage defects where there is a possibility of a crack or fissure in the bone below.

Brief Summary Text (29):
Angiogenic Factor--as used herein, refers to any peptide, polypeptide, protein or any other compound or composition which induces or stimulates the formation of blood vessels and associated cells (such as endothelial, perivascular, mesenchymal and smooth muscle cells) and blood vessel-associated basement membranes. In vivo and in vitro assays for angiogenic factors are well-known in the art [e.g., Gimbrone, M. A., et al., J. Natl. Cancer Inst., 52, pp. 413-419 (1974); Klagsbrun, M. et al., Cancer Res., 36, pp. 110-113 (1976); Gross et al., Proc. Natl. Acad. Sci. (USA), 80, pp. 2623-2627 (1983); Gospodarowicz et al., Proc. Natl. Acad. Sci. (USA), 73, pp. 4120-4124 (1976); Folkman et al., Proc. Natl. Acad. Sci. (USA), 76, pp. 5217-5221 (1979); Zetter, B. R., Nature (London), 285, pp. 41-43 (1980); Azizkhan, R. G. et al., J. Exp. Med., 152, pp. 931-944 (1980)].

Brief Summary Text (30):
Anti-Angiogenic Agent--as used herein, refers to any compound or composition with biological activity that prevents ingrowth of blood vessels from the underlying bone tissue into the cartilage tissue, such as anti-invasive factors, cartilage-derived angiogenesis inhibitors, angiostatin, metalloprotease inhibitors, antibodies against angiogenesis-inducing factors (including bFGF and endothelial cell stimulating angiogenic factor (ESAF)), Suramin (Germanin.RTM., Bayer Co., Germany), fumagillin, fumagillin analogues and AGM-1470 [Peacock, D. J. et al., Cellular Immunology, 160, pp. 178-84 (1995)]. In vivo and in vitro assays to determine anti-angiogenic agents are well-known in the art [e.g., Moses, M. A., Clinical & Exptl. Rheumatology, 11(Suppl. 8), pp. 567-69 (1993); Moses, M. A. et al., J. Cell Bio., 119(2), pp. 475-82 (1992); Moses, M. A. et al., Science, 248, pp. 1408-10 (1990); Ingber, D. et al., Nature, 348(6), pp. 555-57 (1990)].

Brief Summary Text (87):
The remaining cartilage portion of the defect is completely filled with a matrix composition used to stimulate cartilage repair. The composition for cartilage repair comprises a matrix material containing an anti-angiogenic agent and, if desired, a proliferation agent and a chemotactic agent. Anti-angiogenic agents useful in the compositions and methods of this invention include any agents with biological activity capable of inhibiting vascularization. This invention contemplates that the anti-angiogenic agent may comprise one or more molecules capable of inhibiting angiogenesis. The composition used in this step may also contain a transforming factor

packaged in a delivery system and, if appropriate, in free form as well. In the most preferred method of cartilage repair of the invention, the matrix contains an anti-angiogenic factor (in free form and packaged in or associated with a delivery system for sustained release), a proliferation agent, a chemotactic agent (which may be identical to the proliferation agent), and a transforming factor that is packaged in or associated with a delivery system that releases the transforming factor at a time that the repair cells populating the matrix have begun remodeling the intercellular substance. Preferred compositions are described above.

Brief Summary Text (92):
According to one embodiment of the methods of this invention, the surface of the defect is dried by blotting the area using sterile absorbent tissue, and the defect volume is filled with a sterile enzyme solution for a period of 2-10 minutes to degrade the proteoglycans present on the surface of the cartilage and locally within approximately 1 to 2 .mu.m deep from the surface of the defect. Various enzymes may be used, singly or in combination, in sterile buffered aqueous solutions to degrade the proteoglycans. The pH of the solution should be adjusted to optimize enzyme activity.

Brief Summary Text (93):
Enzymes useful to degrade the proteoglycans in the methods of this invention include chondroitinase ABC, chondroitinase AC, hyaluronidase, pepsin, trypsin, chmotrypsin, papain, pronase, stromelysin and Staph V8 protease. The appropriate concentration of a particular enzyme or combination of enzymes will depend on the activity of the enzyme solution.

Brief Summary Text (94):
In a preferred embodiment of this invention, the defect is filled with a sterile solution of chondroitinase AC at a concentration of 1 U/ml and digestion is allowed to proceed for 4 minutes. The preferred concentration of chondroitinase AC is determined according to the procedure described in Example 1. Any other enzyme used should be employed at a concentration and for a time period such that only superficial proteoglycans down to a depth of about 1-2 .mu.m are degraded.

Brief Summary Text (95):
The amount of time the enzyme solution is applied should be kept to a minimum to effect the degradation of the proteoglycans predominantly in the repair area. For chondroitinase ABC or AC at a concentration of 1 U/ml, a digestion period longer than 10 minutes may result in the unnecessary and potentially harmful degradation of the proteoglycans outside the defect area. Furthermore, digestion times longer than 10 minutes contribute excessively to the overall time of the procedure. The overall time for the procedure should be kept to a minimum especially during open arthrotomy, because cartilage may be damaged by exposure to air [Mitchell et al., (1989), supra]. For these reasons, in the embodiments of the methods of this invention that include the step of degradation of proteoglycans by enzymatic digestion, digestion times of less than 10 minutes are preferred and digestion times of less than 5 minutes are most preferred.

Brief Summary Text (96):
According to the methods of this invention, after the enzyme has degraded the proteoglycans from the surface of the defect, the enzyme solution should be removed from the defect area. Removal of the enzyme solution may be effected by using an aspirator equipped with a fine suction tip followed by sponging with cotonoid. Alternatively, the enzyme solution may be removed by sponging up with cotonoid alone.

Brief Summary Text (97):
Following removal of the enzyme solution, the defect should be rinsed thoroughly, preferably three times, with sterile physiologic saline (e.g., 0.15M NaCl). The rinsed defect site should then be dried. Sterile gauze or cottonoid may be used to dry the defect site.

Brief Summary Text (98):
The adhesion of the matrix to the cartilage of the defect can also be enhanced by using fibrin glue (i.e., blood factor XIII or fibrin stabilization factor) to promote chemical bonding (cross-linking) of the fibrils of the matrix to the cartilage collagen fibrils on the defect surface [see Gibble et al., Transfusion, 30(8), pp.

741-47 (1990)]. The enzyme transglutaminase may be used to the same effect [see e.g., Ichinose et al., J. Biol. Chem., 265(23), pp. 13411-14 (1990); "Transglutaminase," Eds: V. A. Najjar and L. Lorand, Martinus Nijhoff Publishers (Boston, 1984)]. Other compounds that can promote adhesion of extracellular materials may also be used.

Detailed Description Text (3):
Enzyme Testing for Proteoglycan Removal

Detailed Description Text (4):
In order to promote and improve matrix adherence along superficial defect surfaces of articular cartilage tissue, proteoglycan molecules within the superficial cartilage matrix may be removed enzymatically, in order to expose the collagen fibrillar network to externally applied matrices and to migrating repair cells. Various proteases and glycosaminoglycan-degrading enzymes are suitable to be used for this purpose, but pH conditions should be controlled to provide maximal activity for each enzyme.

Detailed Description Text (5):
In this example, we tested chondroitinase ABC (0.5-5 U/ml) and trypsin (0.5-4%) for their ability to effect proteoglycan removal. Knee joints from freshly slaughtered rabbits, obtained from a local butcher, were employed. Mechanically-created superficial cartilage defects were exposed to the enzyme solutions for a period of 4 minutes. Solutions were then removed with absorbent tissue and the defect sites rinsed thoroughly with physiologic saline. Following this procedure, cartilage tissue was fixed immediately in 2% (v/v) glutaraldehyde solution (buffered with 0.05M sodium cacodylate, pH 7.4) containing 0.7% (w/v) ruthenium hexamine trichloride (RHT) for histological examination. The post-fixation medium consisted of a 1% RHT-osmium tetroxide solution (buffered with 0.1M sodium cacodylate). Tissue was dehydrated in a graded series of ethanol and embedded in Epon 812. Thin sections were cut, stained with uranyl acetate and lead citrate, and examined in an electron microscope. In these sections, RHT-fixed (i.e., precipitated) proteoglycans appeared as darkly-staining granules. Enzyme concentrations removing a superficial layer of proteoglycans no more than 1-2 .mu.m in thickness were defined as optimal (deeper penetration of enzymes could affect the underlying chondrocytes). Chondroitinase ABC was found to be optimally active at a concentration of approximately 1 U/ml. Trypsin was found to be optimally active at a concentration of approximately 2.5%. The optimal activity range for other glycosaminoglycanases or proteases may be determined in a similar manner. Any buffer may be used in conjunction with the enzyme provided that it is nontoxic and that its maximal buffering capacity occurs at a pH value close to that required for maximal enzyme activity.

Detailed Description Text (8):
The possibility of promoting matrix adhesion along defect surfaces by controlled enzyme digestion of superficial cartilage proteoglycans was investigated. Defects were created in the knee joints of three mature rabbits by cutting with a planing knife. These defects were not enzyme treated. The defects were filled with a fibrin matrix, formed by mixing 20 .mu.l of a thrombin solution (100 U/ml aqueous buffer) with each ml of fibrinogen solution (1 mg/ml aqueous buffer) approximately 200 seconds before filling the defect. The rabbits were sacrificed after 1 month and the knee joints examined to determine the extent to which the fibrin matrix had adhered to the defect site. The results were compared to those achieved in rabbits whose defects had been treated with chondroitinase ABC (1 U/ml for 4 minutes) before the defect was filled with fibrin matrix (see Examples 3, 4 and 5).

Detailed Description Text (9):
The fibrin matrices deposited in defect areas left untreated with an enzyme exhibited low affinity to adhere to the defect surface. Following enzyme treatment, the sticking capacity of the fibrin matrices (determined indirectly by measuring mechanical strength to adhere, i.e., by testing the easiness with which the matrix could be pushed away manually with the tip of a forceps, and indirectly by noting the number of defects in which the matrix successfully remained sticking throughout the experiment) was significantly increased. The low affinity of matrices for the defect surfaces in the absence of enzyme treatment probably is due to a local inhibition of matrix adhesion by proteoglycan molecules and an inhibition of fibrin polymerization. Both of these effects are prevented by enzymatic removal of superficial proteoglycans along the defect surface area.

Detailed Description Text (16):
Each of these factors was applied locally to defects produced in the knee following chondroitinase ABC treatment and rinsing as described in Example 2. A total of ten animals (two per growth factor) were utilized. Each growth factor was able to chemotactically attract or locally stimulate proliferation of repair cells to the defect surfaces sufficiently to completely cover the defect surfaces. However, the cells were only present on the surfaces of the defects, and in no instance was proliferation of the repair cells adequate to fill the defect volume.

Detailed Description Text (31):
In this experiment, a group of six mature rabbits were subjected to knee surgery to produce superficial defects as in Example 2. A full treatment scheme for superficial defect repair was applied, i.e., treatment with chondroitinase ABC (1 U/ml for 4 minutes), followed by filling the defect site with fibrin matrix (1 mg/ml fibrinogen solution, 20 .mu.l 100 U/ml thrombin solution per ml of fibrinogen solution) containing free TGF-.beta. (-2-10 ng/ml) and liposome encapsulated TGF-.beta. (-800 ng/ml). Three rabbits were sacrificed at eight, ten and twelve days postoperatively, the remaining three at twenty, twenty-four and twenty-eight days. Transformation of the primitive, fibroblast-like repair cell tissue into hyaline cartilage tissue occurred between days twelve and twenty in this animal model. This was determined on the basis of histological examination. At days eight to twelve, loose fibrous repair tissue was still present (the applied fibrin matrix being partially or completely remodeled), whereas at day twenty and subsequently, the defect space was partially or completely filled with hyaline cartilage tissue.

Detailed Description Text (34):
The experimental procedures utilized in the rabbit model, supra, were applied to a larger animal model, the mini-pig. Superficial defects (0.6 mm wide, 0.6 mm deep and approximately 10-15 mm long) were created in four mature mini-pigs (2-4 years old, 80-110 lbs.) by cutting with a planing knife in the patellar groove and on the medial condyle. The defects were then treated with chondroitinase ABC (1 U/ml for 4 minutes, as used for rabbits, supra). The enzyme solution was removed, the defect dried, rinsed with physiological saline, then dried again. The defect sites were then filled with a fibrinogen matrix solution. The fibrinogen matrix solution used in this experiment contained 2-6 ng of free TGF-.beta. per ml, and 1500-2000 ng of liposome-encapsulated TGF-.beta. per ml of fibrinogen solution. Prior to filling the defects, thrombin was added to the matrix solution as described above in the rabbit experiment.

Detailed Description Text (38):
Full-thickness articular cartilage defects, 1 mm deep and 10 mm wide, were created in the medial condyles and patellar grooves of adult mini-pig knee joints. Five lesions were effected in each of two animals, using a planing instrument. In each patellar groove, two defects were made in the cranial region, two defects in the caudal region and one defect in the medial femoral condyle. The vertical extensions of each lesion into the subchondral bone (containing blood vessels and bone marrow cells) was controlled macroscopically by the occurrence of bleeding to insure that a full-thickness lesion had been made in the joint. The defects were then treated with chondroitinase AC (1 U/ml for 4 minutes). The enzyme solution was removed, the defect dried, rinsed with physiological saline, then dried again. The defect sites were then filled with a cartilage repair matrix solution. The matrix solution used in this experiment consisted of a copolymer of gelatin (Gelfoam, Upjohn) (used at 100 mg per ml) and fibrinogen (used at 20 mg per ml). Thrombin (used at 50 I.U.) was added to the top surface of the defect after the matrix was placed in the defect and was allowed to diffuse into the matrix.

Other Reference Publication (1):
Coomber, "Suramin Inhibits C6 Glioma-Induced Angiogenesis In Vitro," J. Cell. Biochem., 58, pp. 199-207 (1995).

Other Reference Publication (8):
Ingber et al., "Synthetic Analogues Of Fumagillin That Inhibit Angiogenesis And Suppress Tumor Growth," Nature, 348, pp. 555-557 (1990).

Other Reference Publication (10):

Kuettner et al., "Morphological Studies On The Resistance Of Cartilage To Invasion By Osteosarcoma cells in Vitro And In Vivo," Cancer Research, 36, pp. 277-287 (1975).

Other Reference Publication (11):
Langer, "Drug Delivery Systems For Angiogenesis Simulators & Inhibitors," J. Cell. Biochem., 16A, p. 39, abstract CA 023 (1992).

Other Reference Publication (17):
Peacock et al., "A Novel Angiogenesis Inhibitor Suppresses Rat Adjuvant Arthritis," Cellular Immunology, 160(2), pp. 178-184 (1995).

CLAIMS:

16. The method of claim 15 wherein the agent to degrade proteoglycans is chondroitinase AC.